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Ecofriendly management of fungal antagonistic *Trichoderma* sp. against charcoal rot of sunflower caused by *Macrophomina phaseolina* (Tassi) Goid.

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ABSTRACT

Sunflower *(Helianthus annus L.)* is one of the most important oil seed crops grown all over the India. Charcoal rot caused by *Macrophomina phaseolina* is a major disease causing severe yield loss upto 52 per cent.The pathogen invasion occurs from the seedling to maturity stage. To overcome these problem *In vitro*, sensitivity of *M. phaseolina* determined through inhibition zone technique to seven isolates offungi antagonistic *viz*.,Tv_1, ETv_2, $EDTv_3$, ATv_4, CTv_5, MTv_6andKTv_7amended into PDA medium on seven days after inoculation. All the antagonists reduced the colony growth of *M. phaseolina* significantly compared to the control. Among this isolates Tv1 was effective (74.12%) in reducing the colony growth of *M. phaseolina* followed byCTv_5(68.39), $EDTv_3$ (48.36)respectively over control.

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INTRODUCTION

Sunflower (*Helianthus annuus*L.) is an oilseed crop characterized with its short growing period, high yield potential, wide range of growing season, low water requirements, wide adaptability to soil condition and its high content (over 40%) of good edible oil (Weiss, 2000).*M. phaseolina* has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species (Indera *et al.,* 1986).The fungus is reported to be soil, seed and stubble borne. The evidence suggests that it is primarily a root inhibiting fungus and produces tuber or cushion shaped 1-8 mm diameter black sclerotia. These sclerotia serve as a primary means of survival (Smith, 1969; Mirza, 1984; Kaisar *et al*., 1988). Manczinger *et al*. (2002) reported that *T. harzianum*, *T. viride*and *T. polysporum* have a strong antagonistic against soil borne pathogens. Rettinassababady *et al.* (2002) reported that the *T. viride* reduced the per cent disease incidence of *M. phaseolina* in blackgram, throughout the crop growth under pot and field.Kumari *et al*. (2012) reported that *Trichoderma harzianum* was found more effective as compared to other biocontrol agents and inhibited maximum fungal growth of *M. phaseolina* followed by *Trichoderma viride u*nder pot conditions. Nagamani and Reddi Kumar (2011) reported that maximum reduction in the growth of *Macrophomina* was observed in the presence of native *Trichoderma* isolate, TW17 to an extent of 62.2 per cent. The objective of the present studies was to study the effect of against with fungal biocontrol agents in the control of sunflower root rot disease caused by *M. phaseolina*.

MATERIALS AND METHODS

Isolation of pathogen (*M. phaseolina*)

The root rot pathogen *M. phaseolina* was isolated from the diseased stems and roots of sunflower collected from different places of Tamil Nadu. The pathogen was isolated and purified. These isolates were maintained in PDA slants and sand maize media for further studies. The composition of PDA medium is presented (Rangaswami, 1993).

Isolation of antagonists

Antagonistic fungi were isolated from the rhizosphere soil collected from different sunflower growing areas of Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. Ten gram of rhizosphere soil was transferred to 250 mL Erlen Meyer flask containing 100 ml of sterile

distilled water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method. From the final dilutions of $10^{-3}$and 10^{-4}, one ml of each aliquot was pipetted out, poured in sterilized Petri dish containing *Trichoderma* special medium (TSM), they were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at room temperature (28 ± 2^0C) for 5 days. Colonies with characteristics of *Trichoderma* spp. was isolated from TSM medium and the culture purified in PDA medium. The pure cultures were maintained on the respective agar slants at 4^oC.

Screening of the fungal antagonists

Seven isolates of *Trichoderma viride* were screened against *M. phaseolina*. *Trichoderma spp*. were placed opposite to each other near the periphery of the Petri plate and incubated at room temperature (28 ± 2^oC). After four days of incubation, mycelia growth of the pathogen and inhibition zone was measured in treated as well as control plates. Per cent inhibition (PI) of mycelia growth was calculated using the formula suggested by Pandey*et al.* (2000).

$$PI = \frac{De-Dt}{De} \times 100$$

De-average diameter of fungal growth (cm) in control

Dt-average diameter of fungal growth (cm) in treatment

The overgrowth of antagonists over the pathogen was measured seven days after incubation. The overgrowth and zone of inhibition was measured and expressed in cm.

Statistical analysis

The pot culture and laboratory experiments were conducted by following Completely Randomized Design (CRD). The field experiment was laid out in Randomized Block Design (RBD). The statistical analysis of the experiment was done by following the methods suggested by Gomez and Gomez (1984). Per cent values were transformed by arcsine or square root transformation.

RESULTS AND DISCUSSION

Survey and Isolation of pathogen

Survey was conducted during 2012-2013 at seven locations in Tamil Nadu. The root rot pathogen *M. phaseolina* was isolated from the diseased stems and roots of sunflower collected from different places of Tamil Nadu. The pathogen was isolated and purified. These isolates were maintained in PDA slants and sand maize media for further studies (Table. 1). The isolates were named based on its location viz., ER_1, EDI_2, APK_3, CO_4, MDU_5 and KPT_6. Isolation of the fungus was made by following standard isolation procedure and the fungus was confirmed with respect to the morphological characters described by Ashby (1927) and Goidanich (1947) as *M. phaseolina*.

Morphological variability was also been reported by many workers in terms of growth, colour and pycnidium production among different isolates of *M. phaseolina*on different hosts (Dhingra and Sinclair, 1973, 1978; Pearson *et al.,* 1986; Atiq*et al.,* 2001; Riaz*et al.,* 2007) which corroborated our findings.

Table 1. Survey and isolation of sunflower root rot caused by *Macrophomina phaseolina* in Tamil Nadu

Location	Districts	Isolate	Per cent Disease incidence
Erur	Perambalur	ER_1	42.20
Ediyar	Ariyalur	EDI_2	38.71
Aruppukottai	Virudhunagar	APK_3	38.99
TNAU	Coimbatore	CO_4	52.00
AC & RI	Madurai	MDU_5	42.00
Kovilpatti	Thoothukudi	KPT_6	45.66
(P=0.05)		**CD**	**1.80**

Isolationof biocontrol agents

During survey program different isolates of fungal and bacterial bio agent were isolated from rhizosphere soil of sunflower plant collected from different location. The details of biocontrol agents isolated for this study were tabulated in Table 2.

Inhibitory effect of *T. viride*on

All the seven isolates of *T. viride* were antagonistic to *M. phaseolina* (Fig.1). All the antagonists reduced the colony growth of *M. Phaseolina* significantly compared to the control. Among this isolates Tv1 was effective (74.12%) in reducing the colony

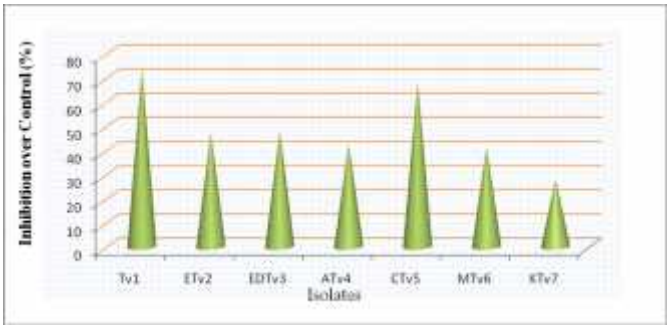
growth of *M. Phaseolina* followed by CTv_5(68.39), $EDTv_3$ (48.36) respectively over control. The control Petri dish without any bio agent received maximum mycelial growth of 8.89 cm in seven days after inoculation.High reduction of pathogen growth in tests was observed by all antagonists.

Table 2. Biocontrol agents screened against *M. phaseolina* under sunflower.

Location	Source	Isolates code
Trichodermaviride		
Erur	Rhizosphere	ETv_2
Edaiyar	Rhizosphere	$EDTv_3$
Aruppukottai	Rhizosphere	ATv_4
TNAU	Rhizosphere	CTv_5
Madurai	Rhizosphere	MTv_6
Thoothukudii	Rhizosphere	KTv_7
Tv_1	Department of Plant Pathology, TNAU, Coimbatore	

Penetration, progression, colonization and sporulation of *Trichoderma* isolate on *M. phaseolina* were also observed. Similar results with other fungi have previously been reported (Yedidia *et al.*, 1999; Eteberian *et al.*, 2000; Benitez *et al.*, 2004; Harman *et al.*, 2004). Soil application of talc based formulation of *T. harzianum*, *T. polysporum* and *T. viride* effectively controlled the root rot (*M. phaseolina*) of eggplant under field condition (Ramezani, 2008).Alice and Sundravadana (2012) reported that the various treatments the basal application of *T. viride*(soil application @ 2.5 kg/ha) and two sprays, the first at 45 days after germination and the second at 90 days after germination, recorded the disease incidence of 12 per cent, which accounted for the disease reduction by 60 per cent in (*M. phaseolina*) on *Gloriosa superba* plant.

Fig 1. Effect of *T. viride* on the growth of *M. phaseolina.*



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